Chromatin structure and Gene expression

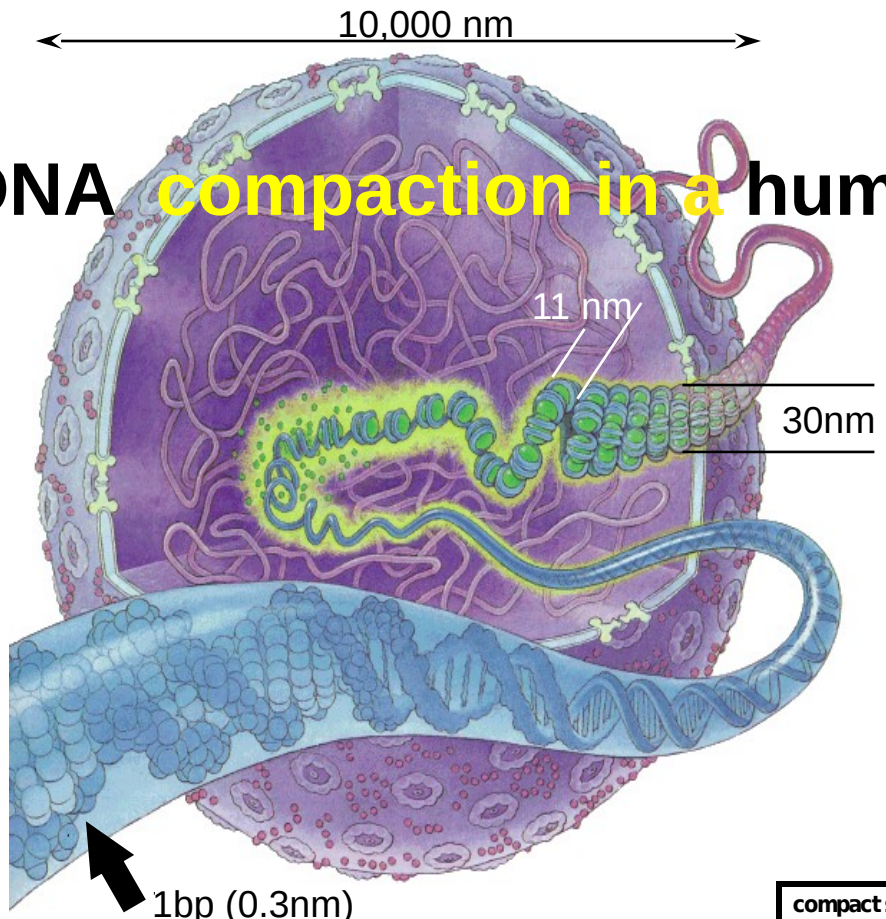
I. Repressive chromatin

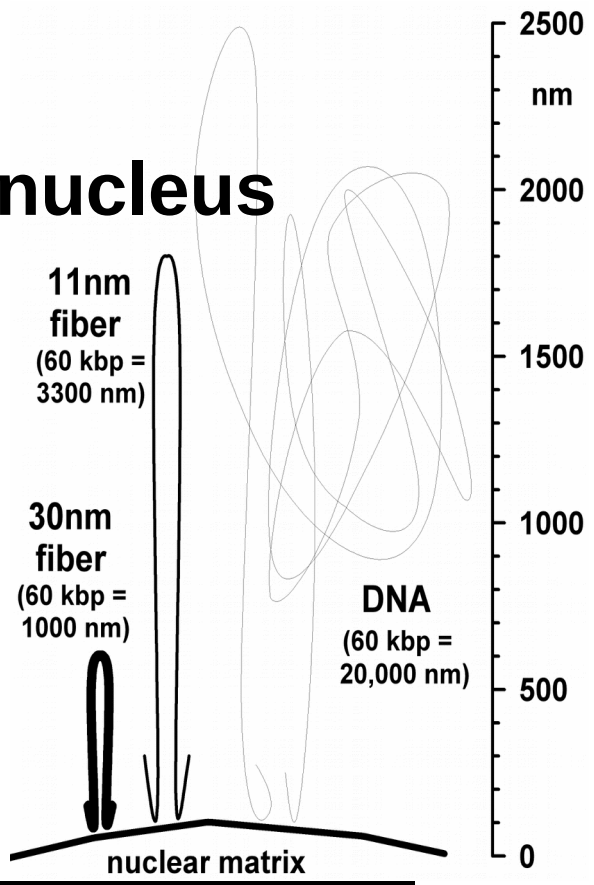
Thursday, 29th October, 2015

Andy Bannister

a.bannister@gurdon.cam.ac.uk

DNA compaction in a human nucleus



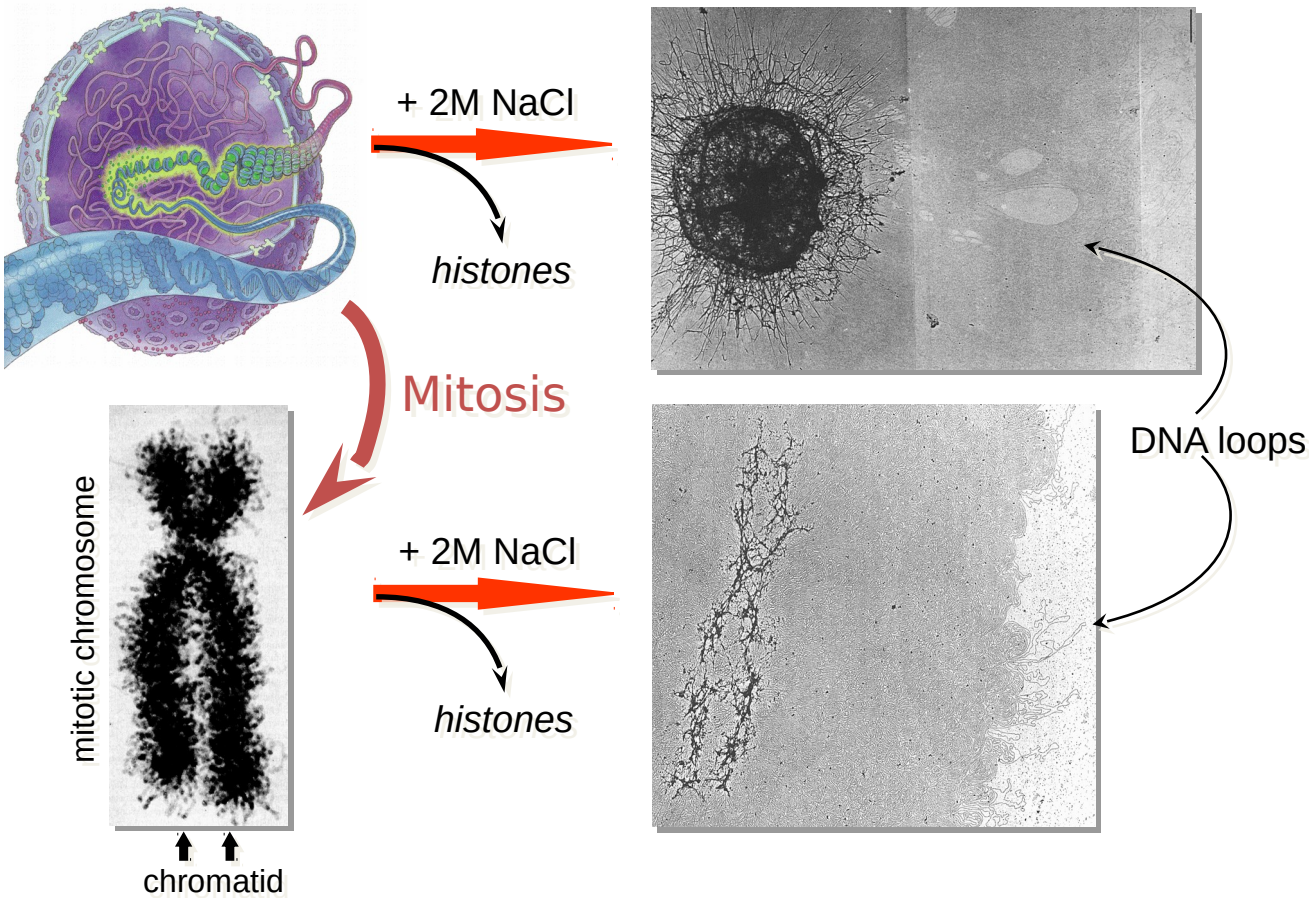


			compact size	DNA	length	compaction
nucleus (human)	2 x 23 = 46 chromosomes	46 DNA molecules	10 µm ball	12,000 Mbp	4 m DNA	**200,000 x**
mitotic chromosome	2 chromatids, 1 µm thick	2 DNA molecules	10 µm long X	2x 130 Mbp	2x 43 mm DNA	**10,000 x**
DNA domain	anchored DNA loop	1 replicon ?	60 nm x 0.5 µm	60 kbp	20 µm DNA	**35 x**
chromatin fiber	approx. 6 nucleosomes per 'turn' of 11 nm		30 nm diameter	1200 bp	400 nm DNA	**35 x**
nucleosome	disk 1 ¾ turn of DNA (146 bp) + linker DNA		6 x 11 nm	200 bp	66 nm DNA	**6 - 11 x**
base pair			0.33 x 1.1 nm	1 bp	0.33 nm DNA	**1 x**

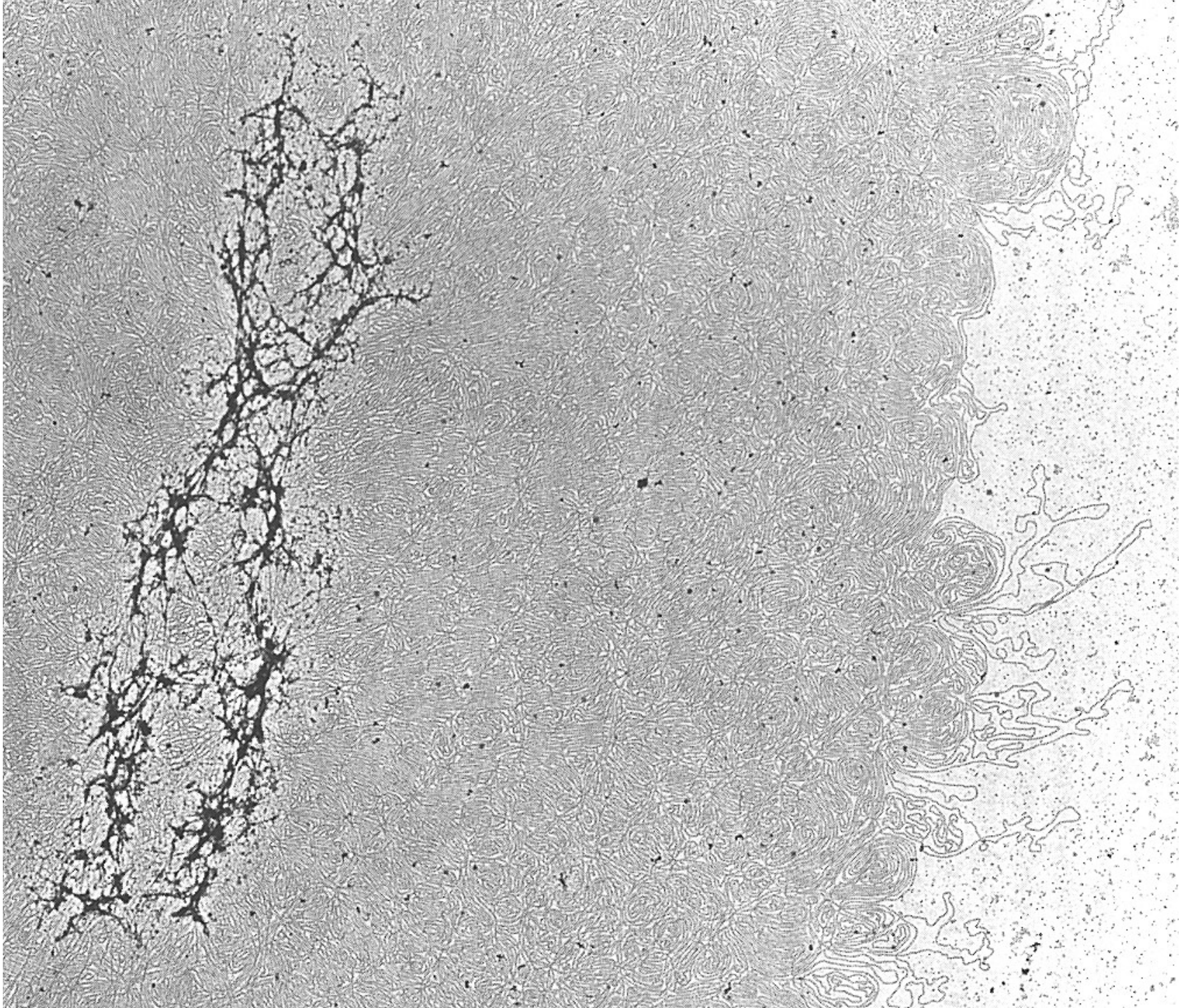
Compaction by chromosome scaffold / nuclear matrix

Compaction of DNA by histones

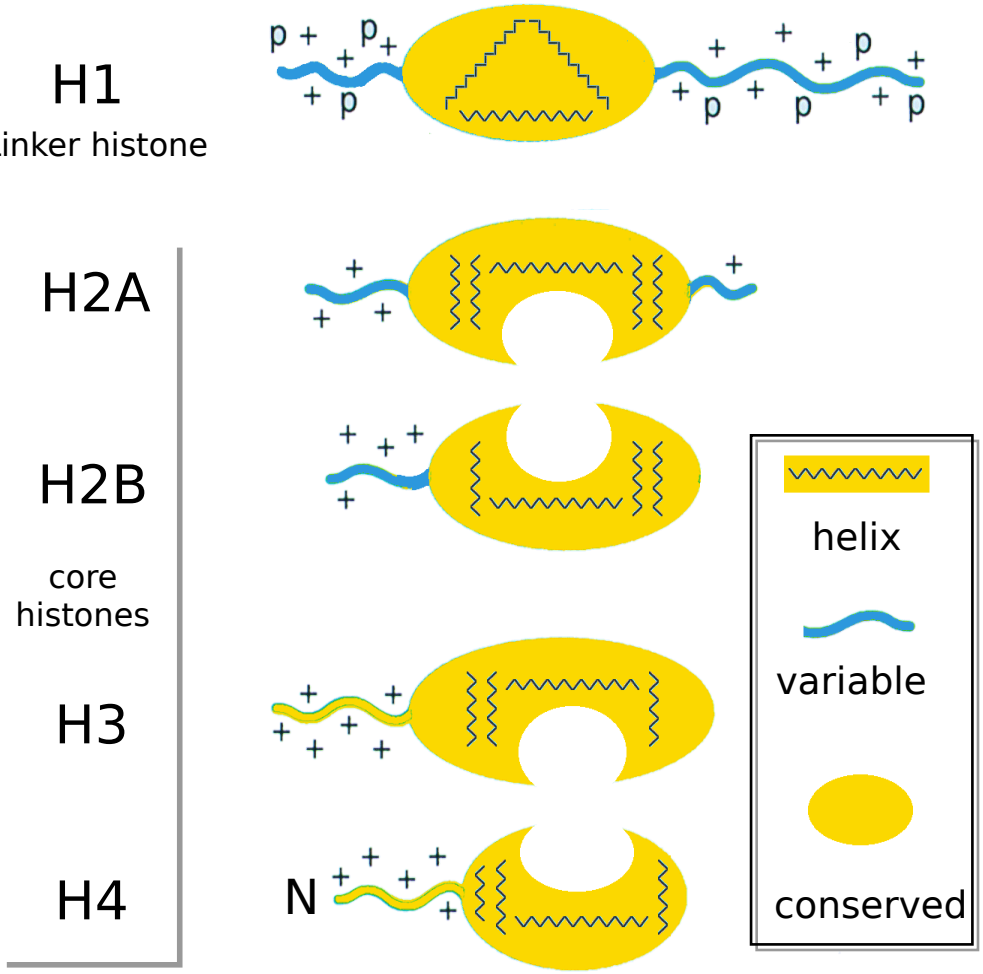
Nuclear - chromosome compaction



The ‘power’ of histones



HISTONES are highly conserved, small, basic proteins



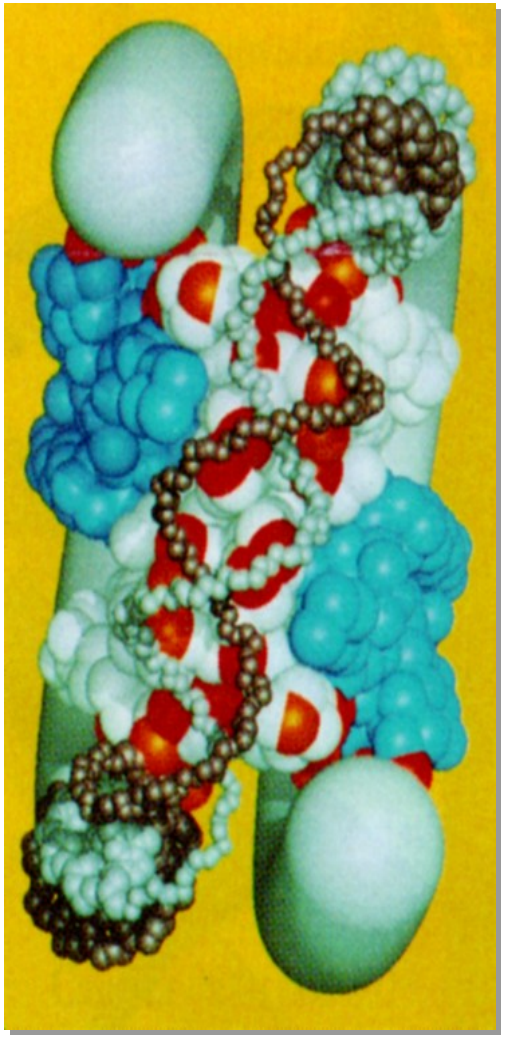
(H3/H4) tetramer binds DNA

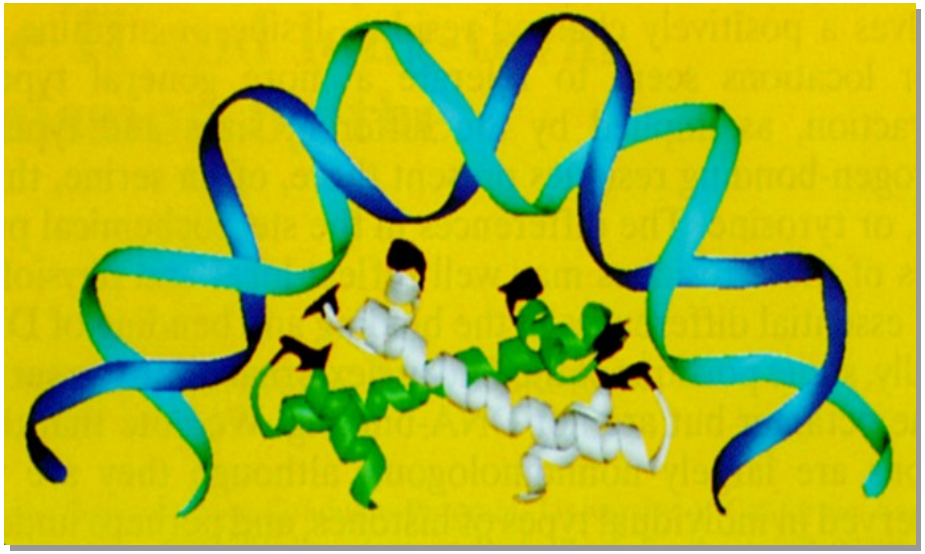
Two (H2A/H2B) dimers bind either side of the tetramer

DNA wraps around a histone octamer to form a nucleosome

Histone Type	Molecular Weight	Number of Amino Acids	Approx. Content of Basic Amino Acids
H1	17,000–28,000	200–265	27% lysine, 2% arginine
H2A	13,900	129–155	11% lysine, 9% arginine
H2B	13,800	121–148	16% lysine, 6% arginine
H3	15,300	135	10% lysine, 15% arginine
H4	11,300	102	11% lysine, 4% arginine

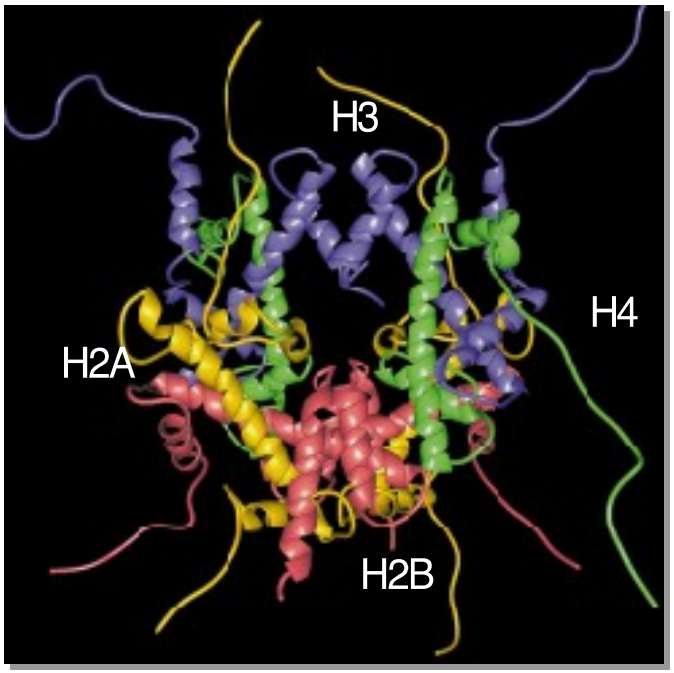
Histone octamer organizes 145bp of DNA: the nucleosome





- Each core histone dimer has 6 DNA binding surfaces that organize 3 DNA turns;
- The histone octamer organizes 145 bp of DNA in 1.75 helical turns of DNA: 48 nm of DNA packaged in a disc 6x11nm

Structure of the nucleosome

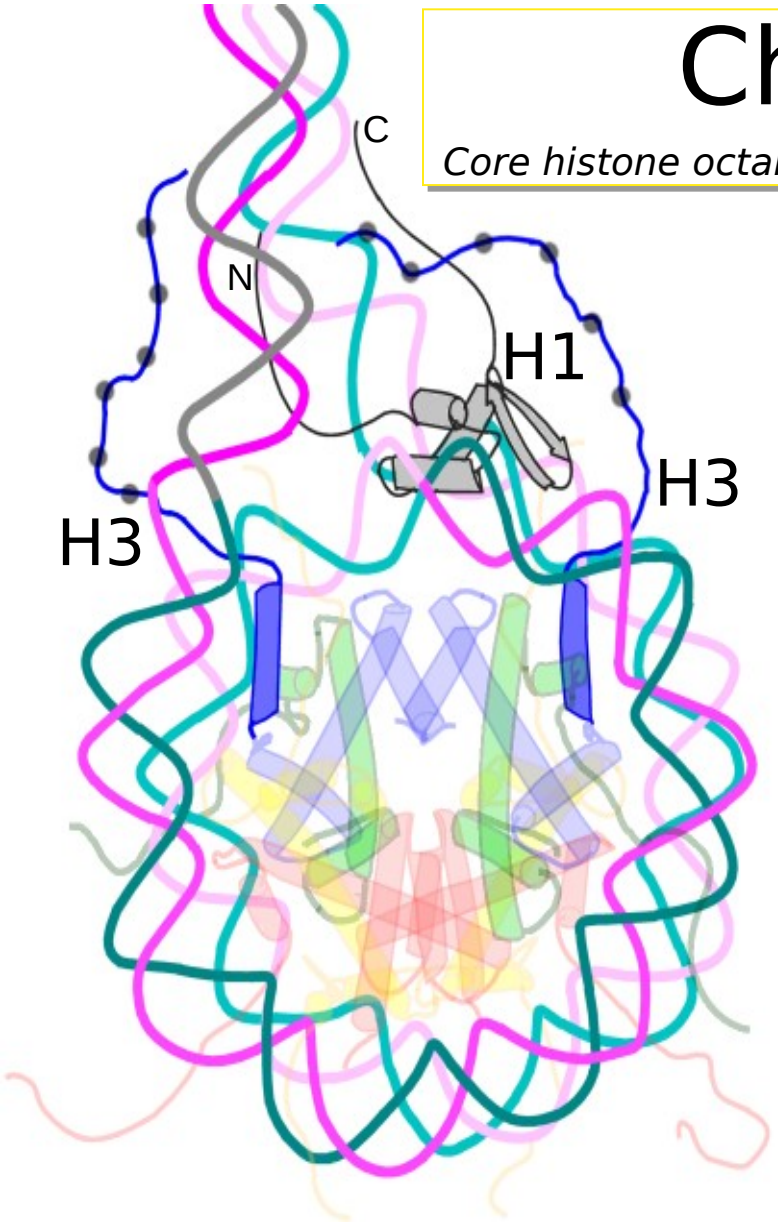


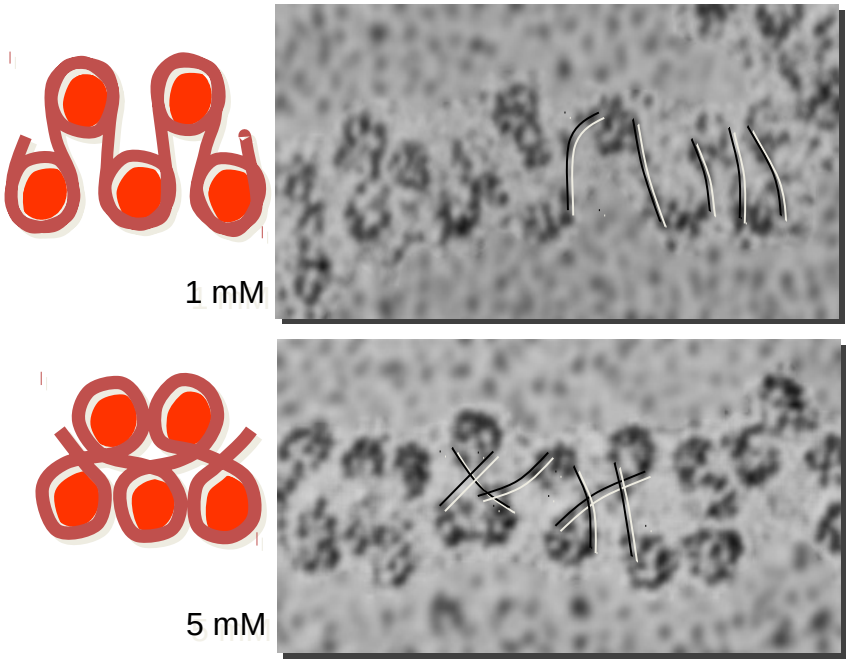
Luger et al., Nature 389, 251-260 (1997)

Helps to understand the structure of higher order chromatin

Chromatosome

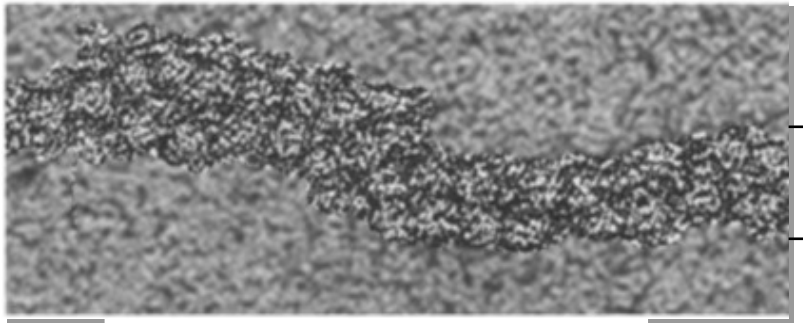
Core histone octamer + 1 Linker Histone + 2 full turns of DNA (168 bp)



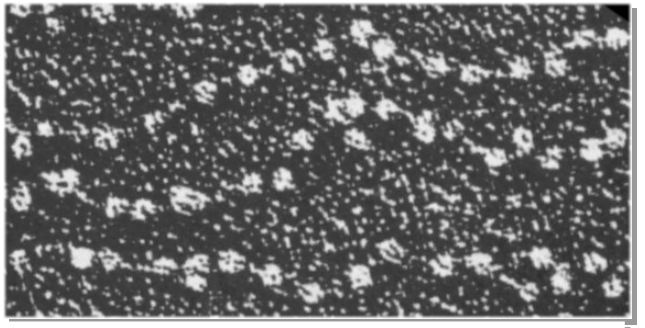


Linker histone H1 and histone termini control linker DNA entry/exit of chromatosome in chromatin fiber

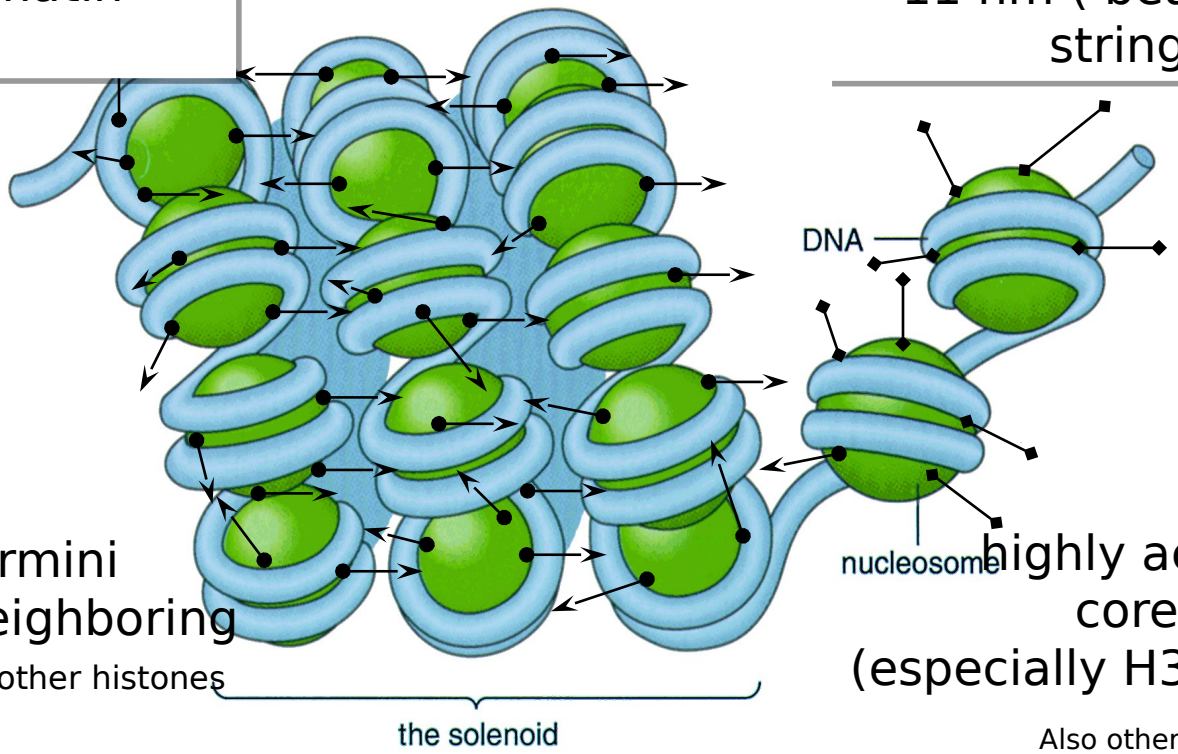
Chromatin fibers



30 nm chromatin fiber



11 nm (‘beads on a string’)



+ charged N termini (bind DNA on neighboring nucleosomes)

and other histones

highly acetylated core histones (especially H3 and H4)

Also other histone mods…….

- HIGH level of histone H1
- Reduced level of histone H1

Increasingly repressive

Chromatin and Gene Expression

Heterochromatin

General Repression

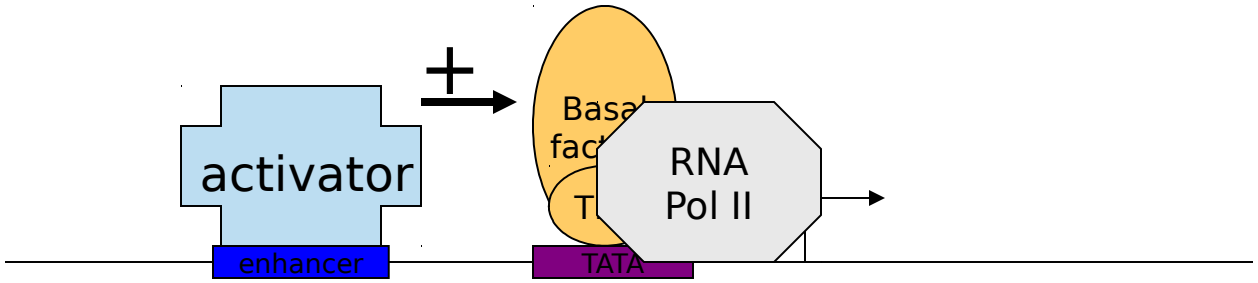
- highly condensed
- inactive
- nr centromeres/telomeres

Euchromatin

Contains Active Genes

- extended structure
- active

Chromatin Derepression leads to transcription



n vitro, **naked** DNA + basal TFs + RNA polII = accurate and efficient transcripti
- stimulated by activator proteins

In vivo, DNA is compacted into nucleoprotein structures (CHROMATIN)

This is highly repressive towards transcription

NEED TO DEREPRESS IN ORDER TO ALLOW EFFICIENT TRANSCRIPTION

- REMODELLING FACTORS (ATP-dependent)
- HISTONE MODIFYING ENZYMES
- FACILITATORS OF ELONGATION

Identification of FACT

(FAcilitates Chromatin Transcription)

Approach

1) Assemble chromatin on promoter template *in vitro* (Gal4 sites)

2) Add activator and allow remodelling

3) Purify remodelled template (gel filtration)

4) Add back purified components or nuclear extract and check

for (i) INITIATION and (ii) ELONGATION

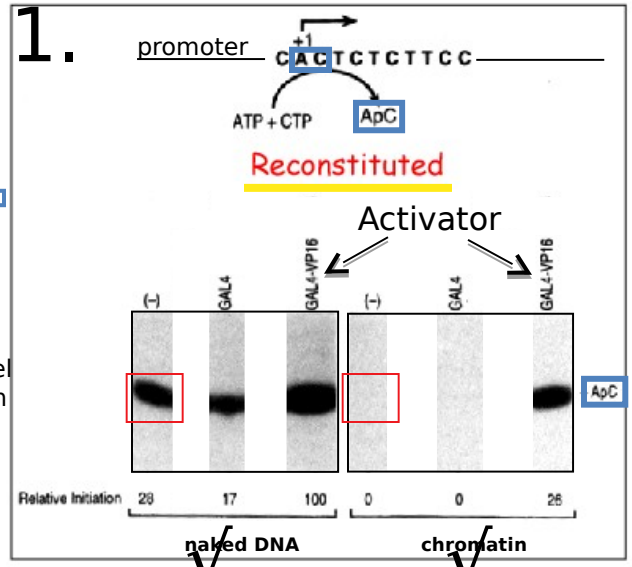
Results

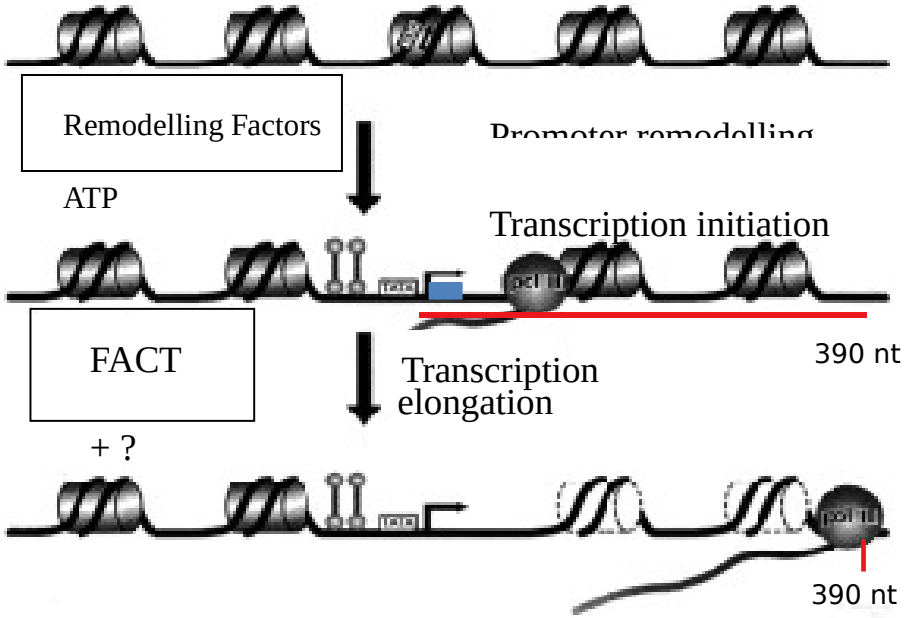
INITIATION

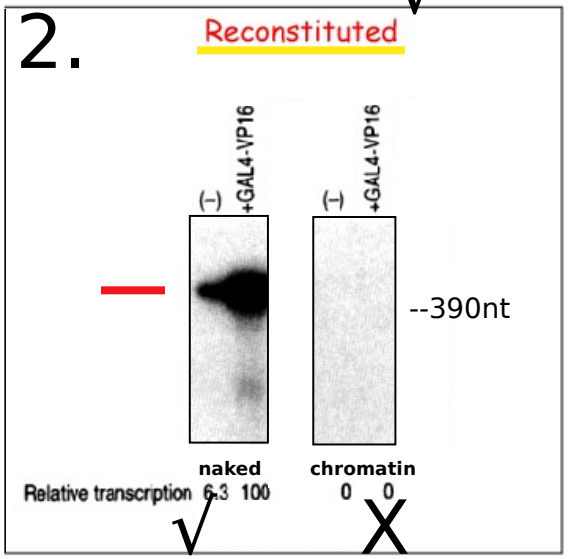
Just add first two nucleotides so that only the first phosphodiester bond is formed

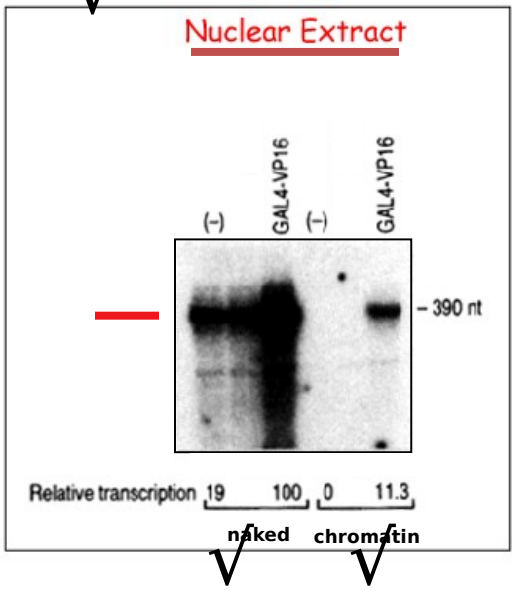
One is RA labeled

Run product on gel and expose to film









ELONGATION

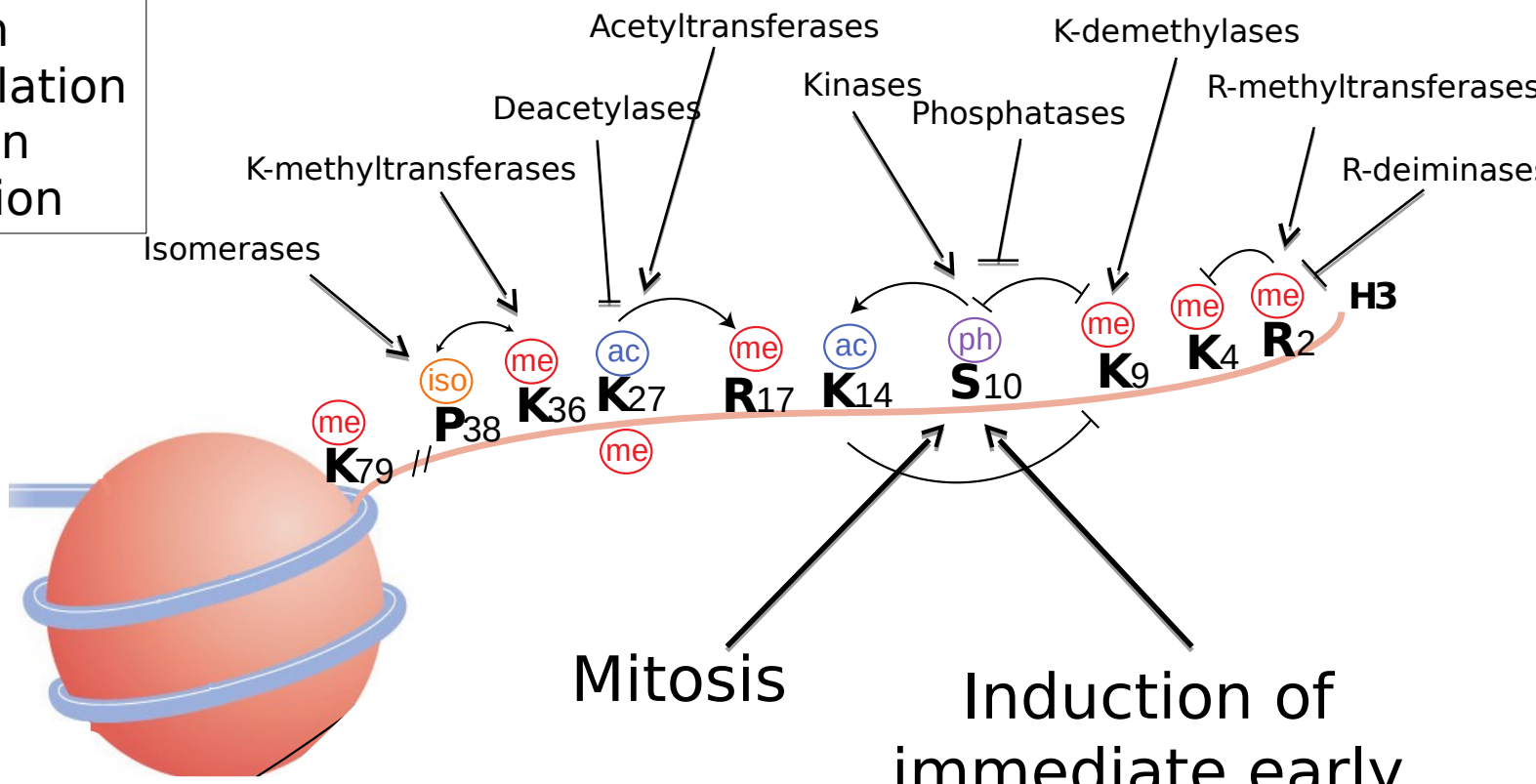
Comparison of 2 ‘systems’

1. Reconstituted: Purified basal TFs,
RNA pol II -/+ Gal4-VP16 (activator)

2. Nuclear Extract Assay in total nuclear extract

Modifications on histone H3 tail

ac: acetylation
ph: phosphorylation
me: methylation
iso: isomerisation

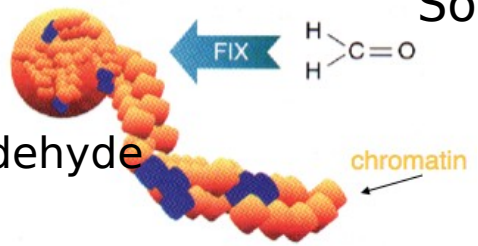


Histone Code Hypothesis

But how do we monitor these modifications *in vivo* at specific loci?

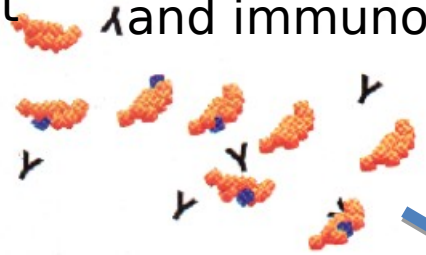
ChIP-seq

x live cells
ith formaldehyde

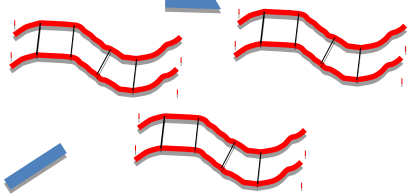


Sonicate/digest

Chromatin purification
and immunoprecipitation

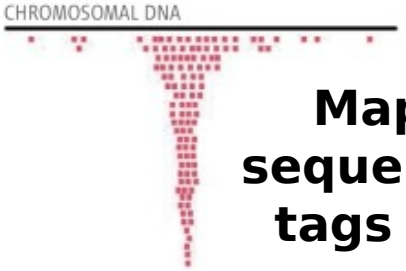


Reverse cross-links
and purify bound DN

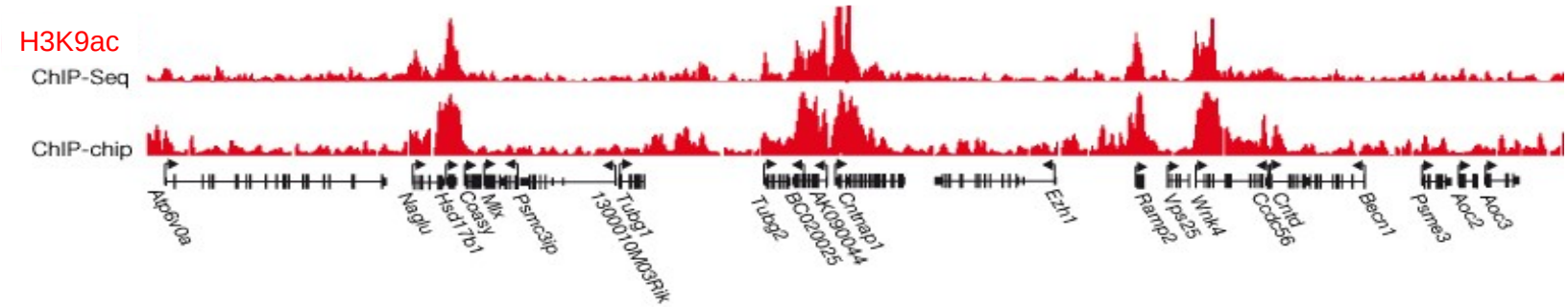


Prepare sequencing library

High-throughput sequencing of DNA ends

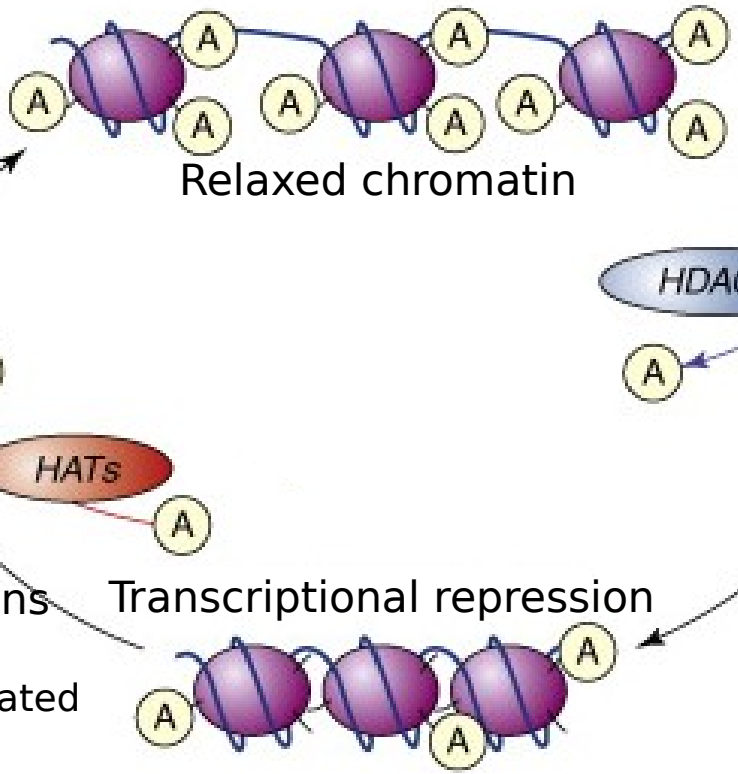


Map sequence tags to genome & identify peaks



Histone acetylation regulates transcription

Transcriptional activation



HDAC examples:

HDAC1

HDAC2

HDAC3

HDAC4 etc……

cetylase examples:

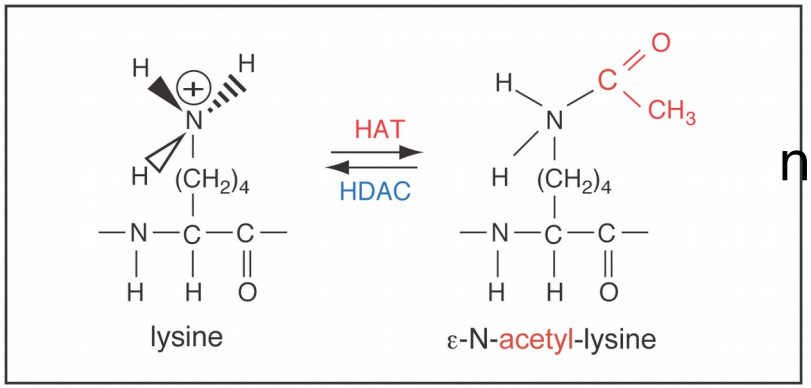
CBP / p300

P/CAF GCN5

TAFii250

} Contain bromodomains

bind acetylated lysines

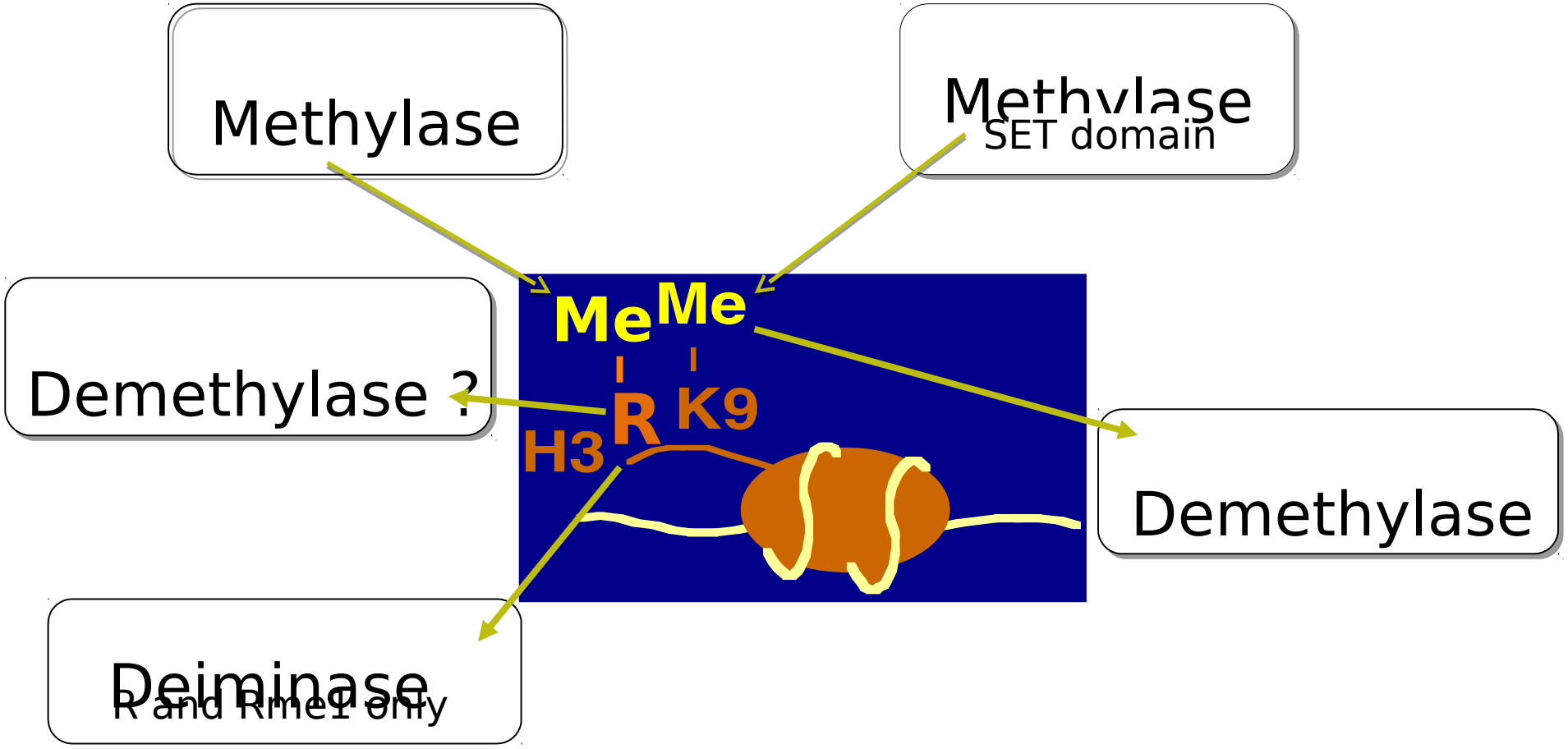


neutralizes lysine's positive charge

Kac functions via:

1. Direct mechanism
2. Providing binding platform(s)

Methylation of lysines and arginines within histones affects chromatin structure and transcription



So what do we need for an epigenetic (histone) code?

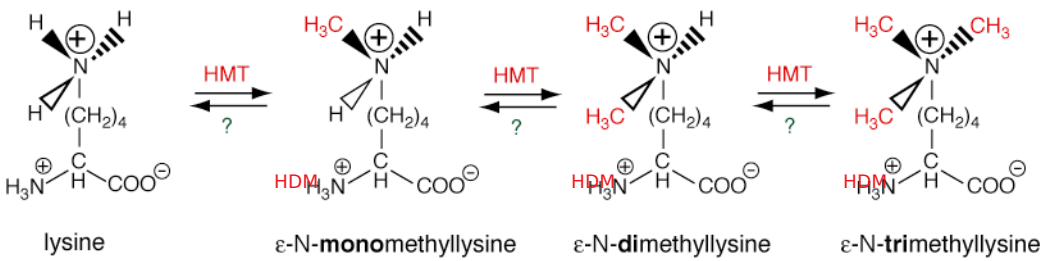
Term	Definition
Writer	An enzyme that introduces a posttranslational modification(s) into a given protein (e.g., a histone methyltransferase).
Eraser	An enzyme that removes a posttranslational modification from a protein (e.g., a histone demethylase).
Reader	A protein or complex that binds specifically to a posttranslationally modified protein, with favorable binding energy contingent upon this modification. Effectors and presenters (see below) are subsets of the more general "reader" category.
Effector	A binding module or domain that binds specifically to a posttranslationally modified substrate, and this binding event recruits other activities contained within the same polypeptide or complex to which it belongs. These recruited activities fall into four general classes: (1) ATP-dependent remodeling of the chromatin fiber, (2) stabilization of a higher order chromatin structure, (3) further posttranslational modification introduction or removal by enzymatic activity (writer or eraser activity), or (4) other gene regulatory effects (e.g., direct recruitment of RNA polymerase II machinery). Effector domains lack enzymatic activity and are analogous to "adaptors" in phosphorylation-dependent signaling pathways (see Seet et al. [2006] for a recent review).
Presenter	A special type of reader module that is able to bind a particular sequence discriminating for a pattern of posttranslational modifications and then present a side chain of this bound epitope for further modification. The presenter serves as accessory factors to writer modules to enable or enhance the activity of the writer on a given substrate.

Let's look at histone methylation as an example……

R and K methylation comes in a number of flavours.....

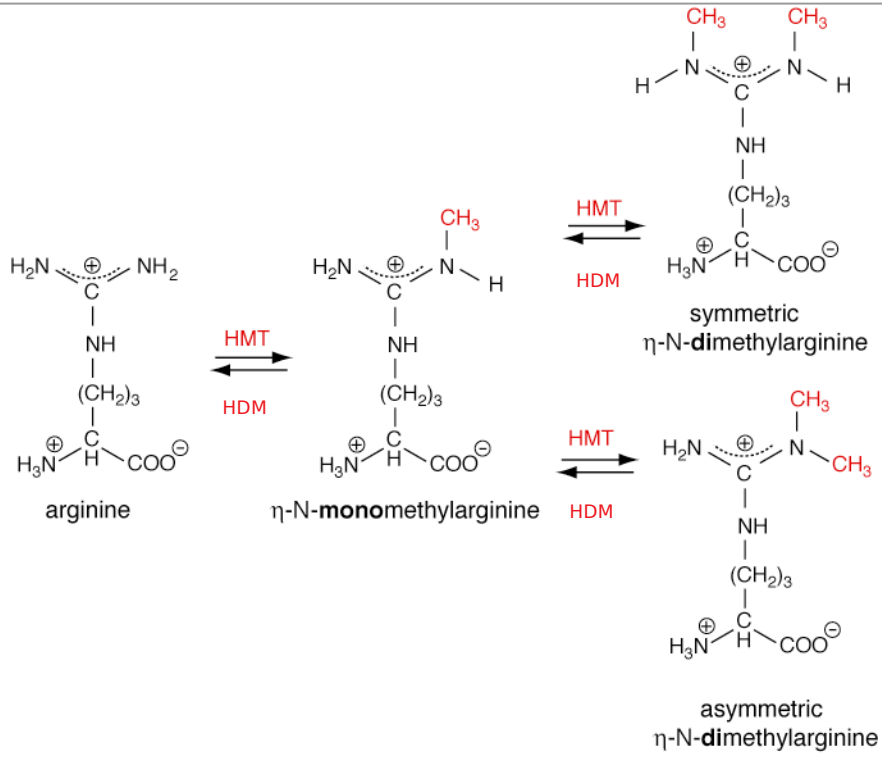
lysine

Kme1, me2, me3



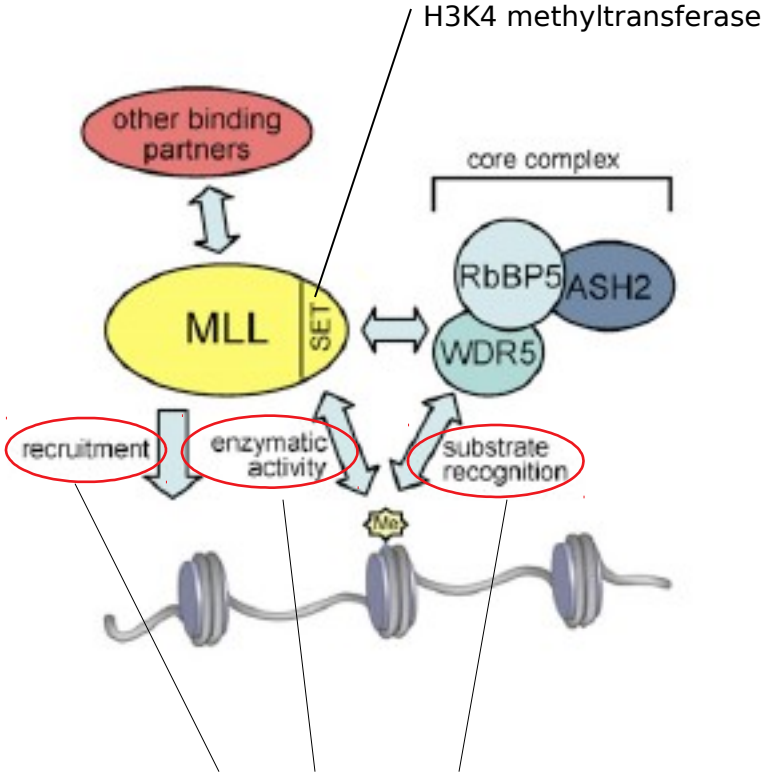
arginine

Rme1, me2as, me2s

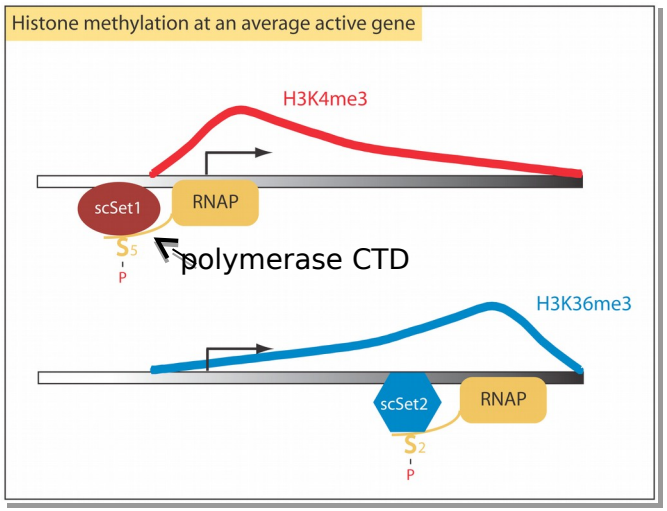


Writing a histone methylation mark

Generally speaking, different histone lysine methylations are associated with different transcriptional activities and they are differentially localized



strongly influenced by complex association

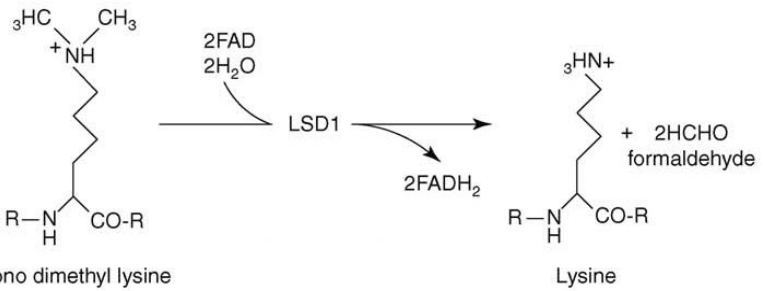


Reversing (**erasing**) histone methylation

(a) Amine oxidation

Mono- and di-methyl lysine only

e.g. LSD1



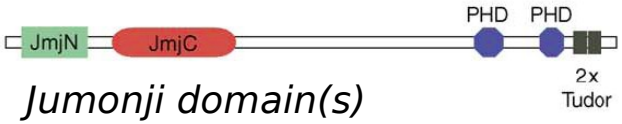
Requires a protonated N in the substrate

(b) Radical attack

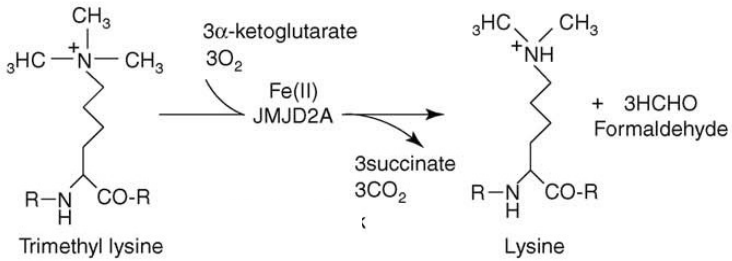
Lysine and arginine (?) substrates:

potentially all methylated forms

e.g. JMJD2A



Jumonji domain(s)



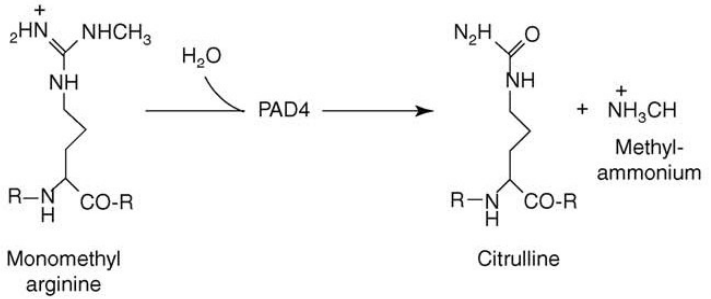
Chemically compatible for me3 removal

but not all enzymes of this class do so

(c) Deimination

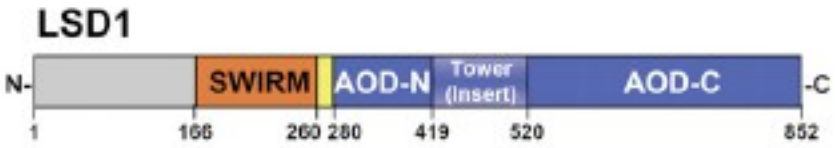
Arginines and mono-methyl R only

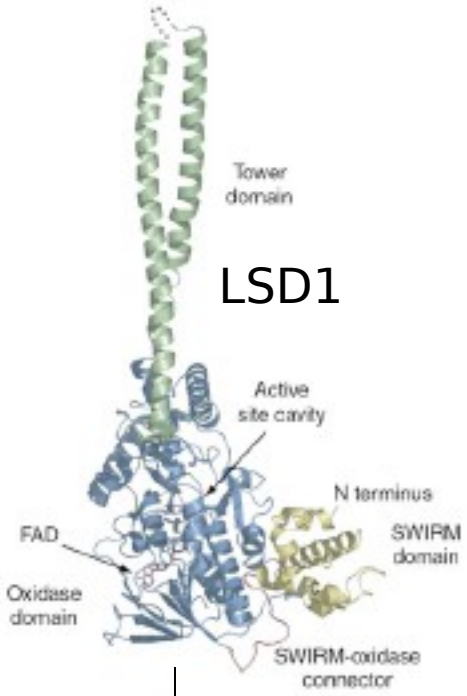
e.g. PADI4



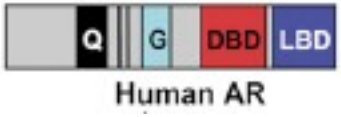
Formation of a non-encoded amino acid

Complex members determine demethylase specificity

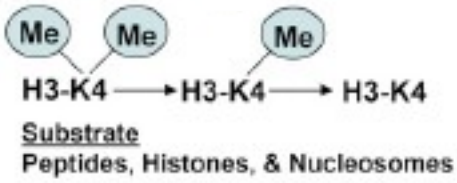




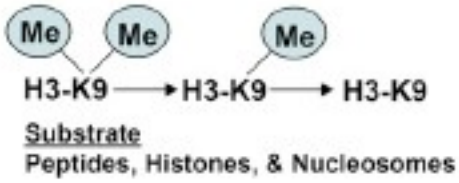
LSD1 forms a complex with either hCo-Rest or hAR



Associated factor determines substrates specificity

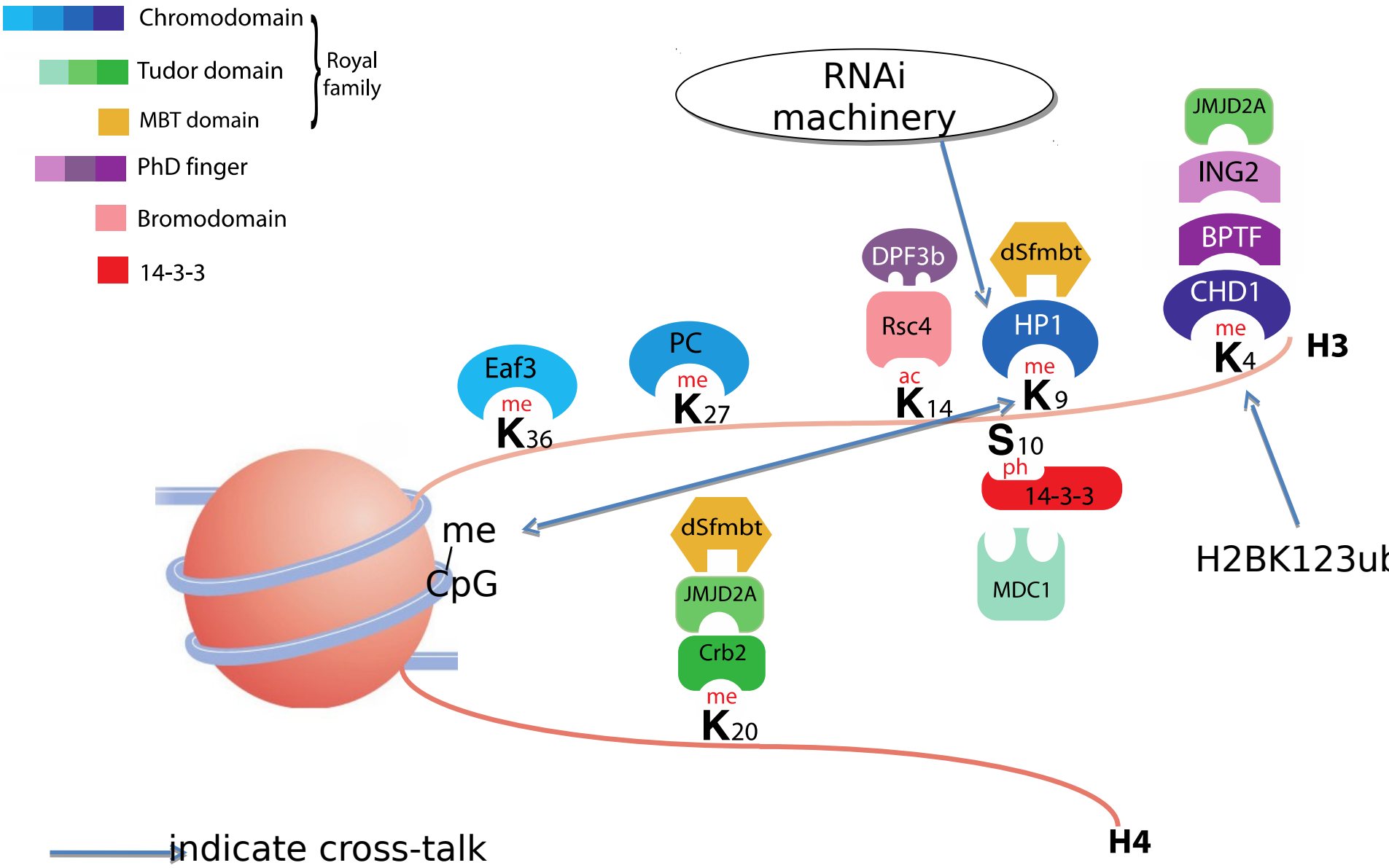


H3K4

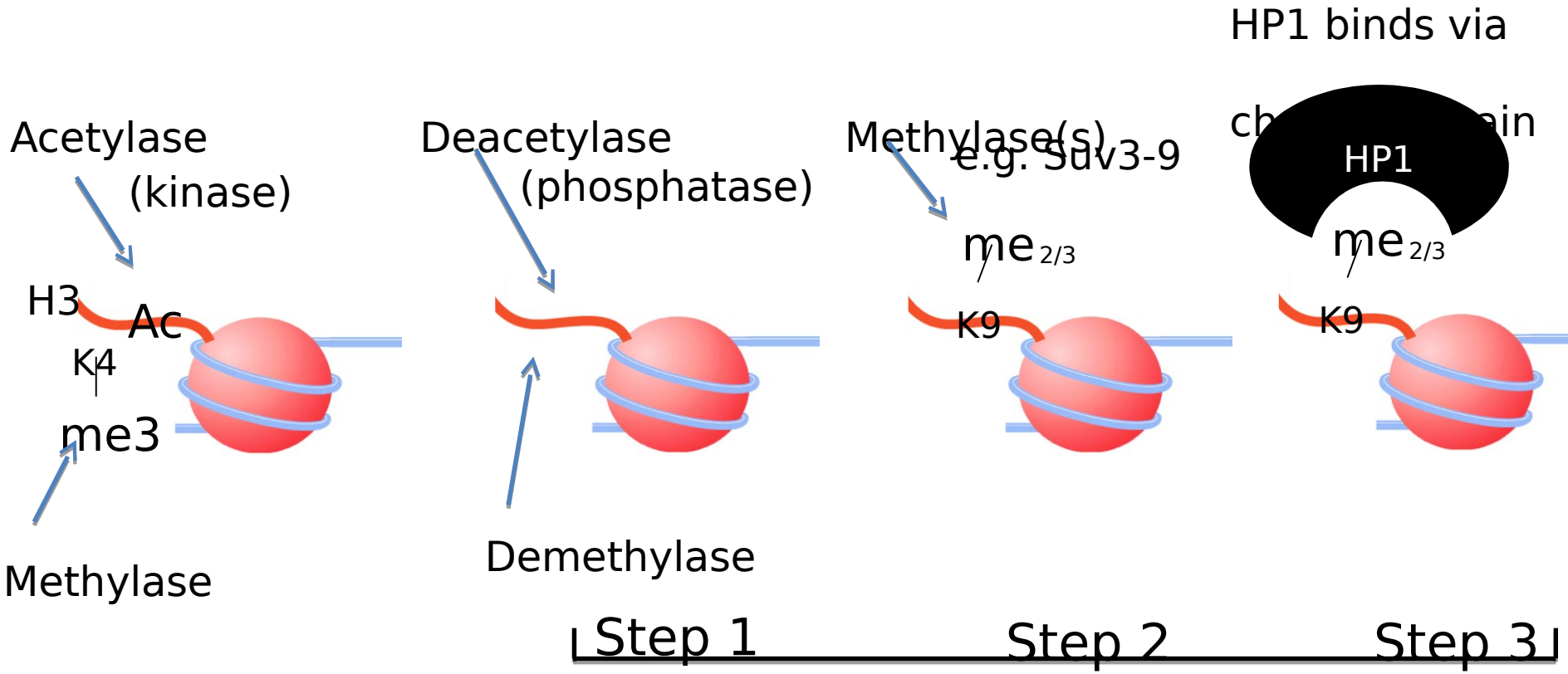


H3K9

Reading the mark



A model for the formation of heterochromatin

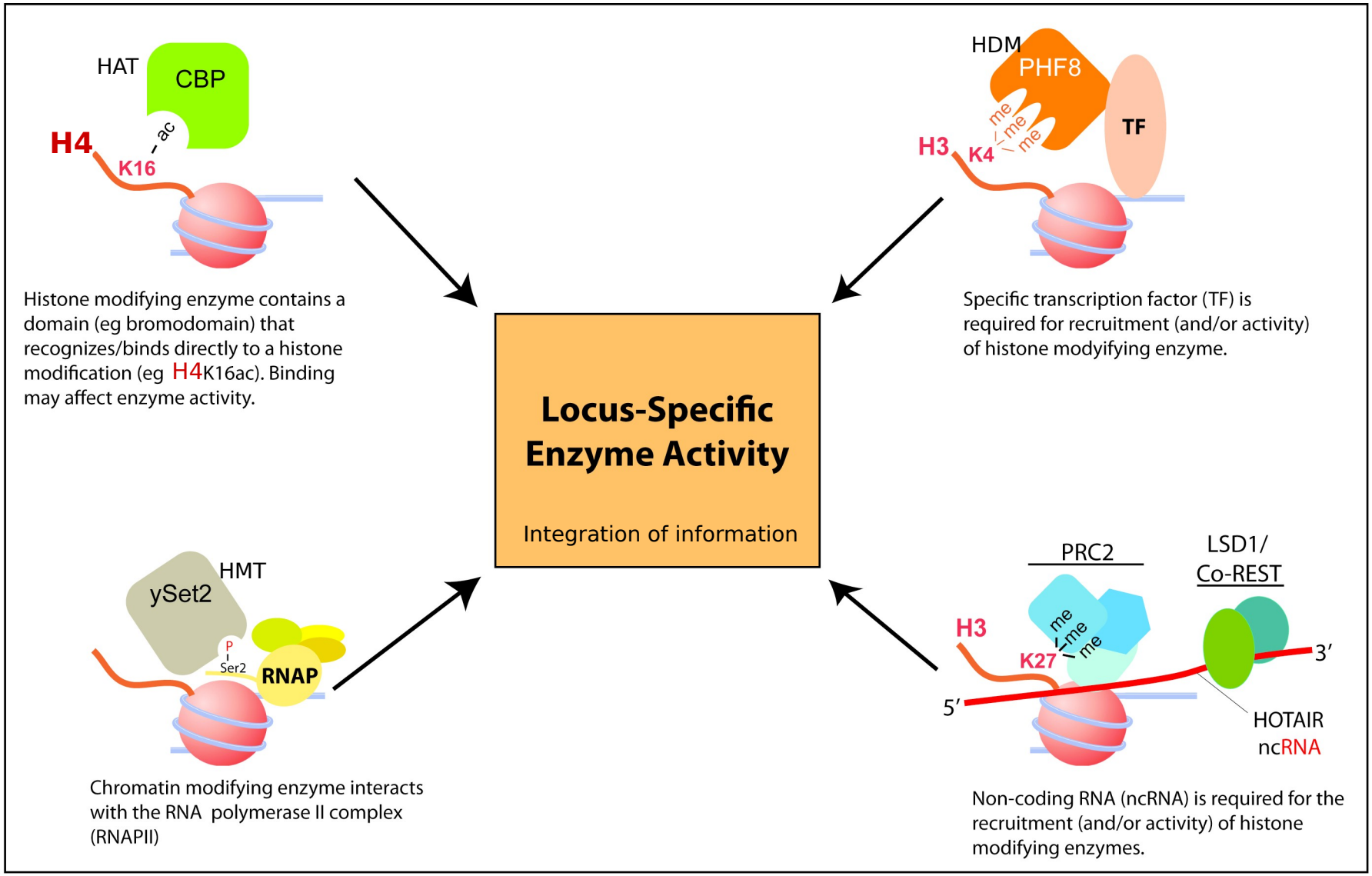


Euchromatin → Heterochromatin

active

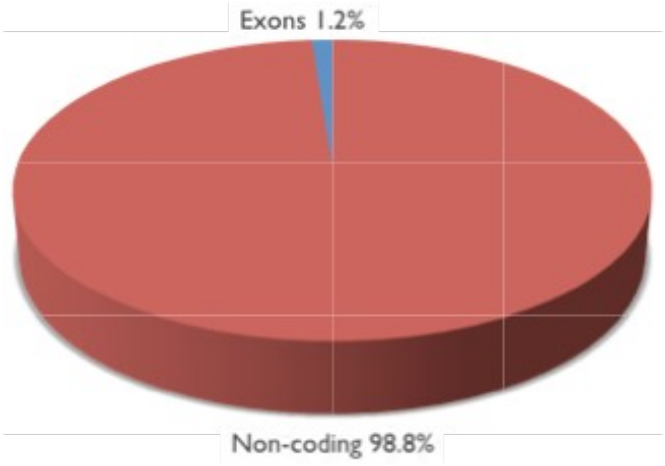
inactive

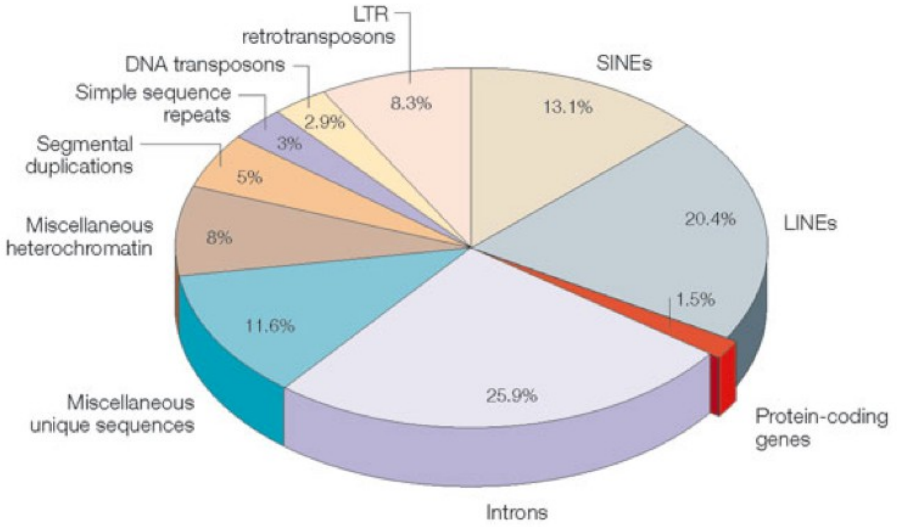
Histone modifying enzyme recruitment mechanisms



Noncoding RNAs are the major output of the human genome

Genome composition





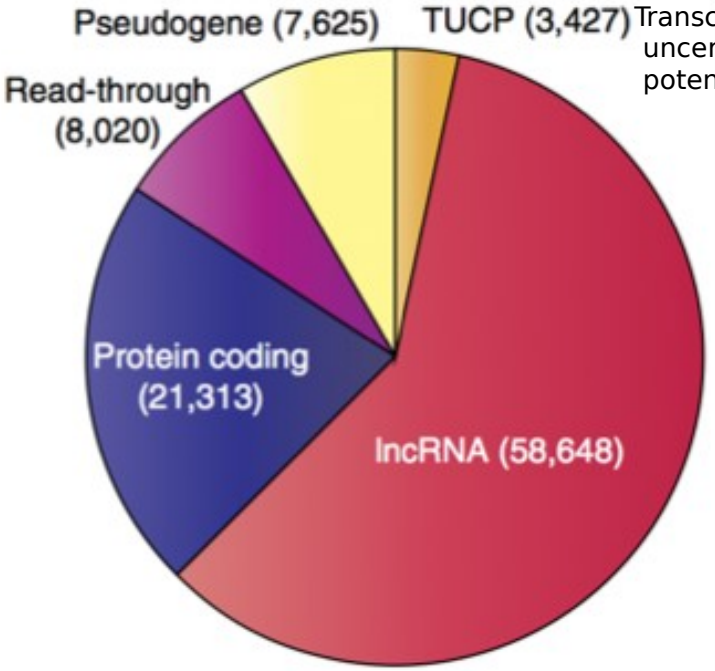
ANALYSIS

nature genetics

Published online 19 January 2015

The landscape of long noncoding RNAs in the human transcriptome

Matthew K Iyer[1,2,11], Yashar S Niknafs[1,3,11], Rohit Malik[1,4], Udit Singhal[1,5], Anirban Sahu[1,4], Yasuyuki Hosono[1], Terrence R Barrette[1], John R Prensner[1], Joseph R Evans[1,6], Shuang Zhao[1,6], Anton Poliakov[1], Xuhong Cao[1,5], Saravana M Dhanasekaran[1,4], Yi-Mi Wu[1], Dan R Robinson[1], David G Beer[6,7], Felix Y Feng[1,6,8], Hariharan K Iyer[9] & Arul M Chinnaiyan[1,2,4,5,8,10]

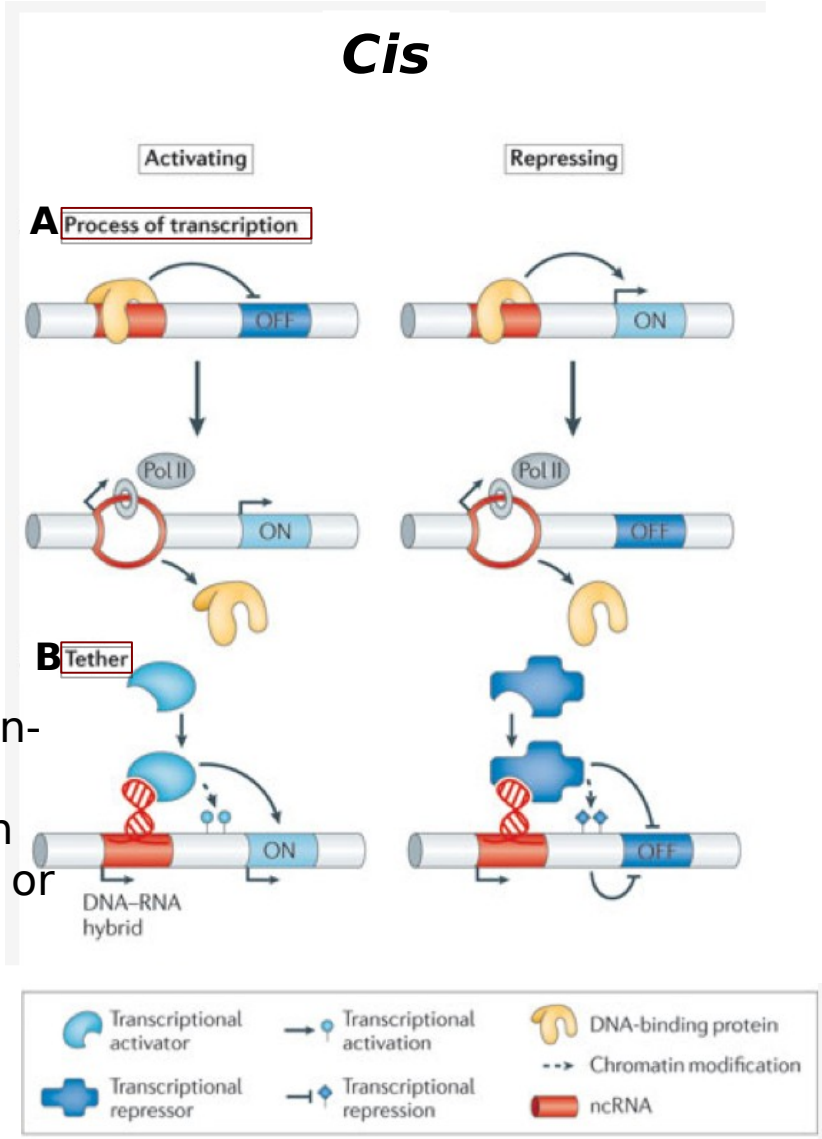


Transcripts of uncertain coding potential

Potential molecular mechanisms of long noncoding RNAs (lncRNAs)

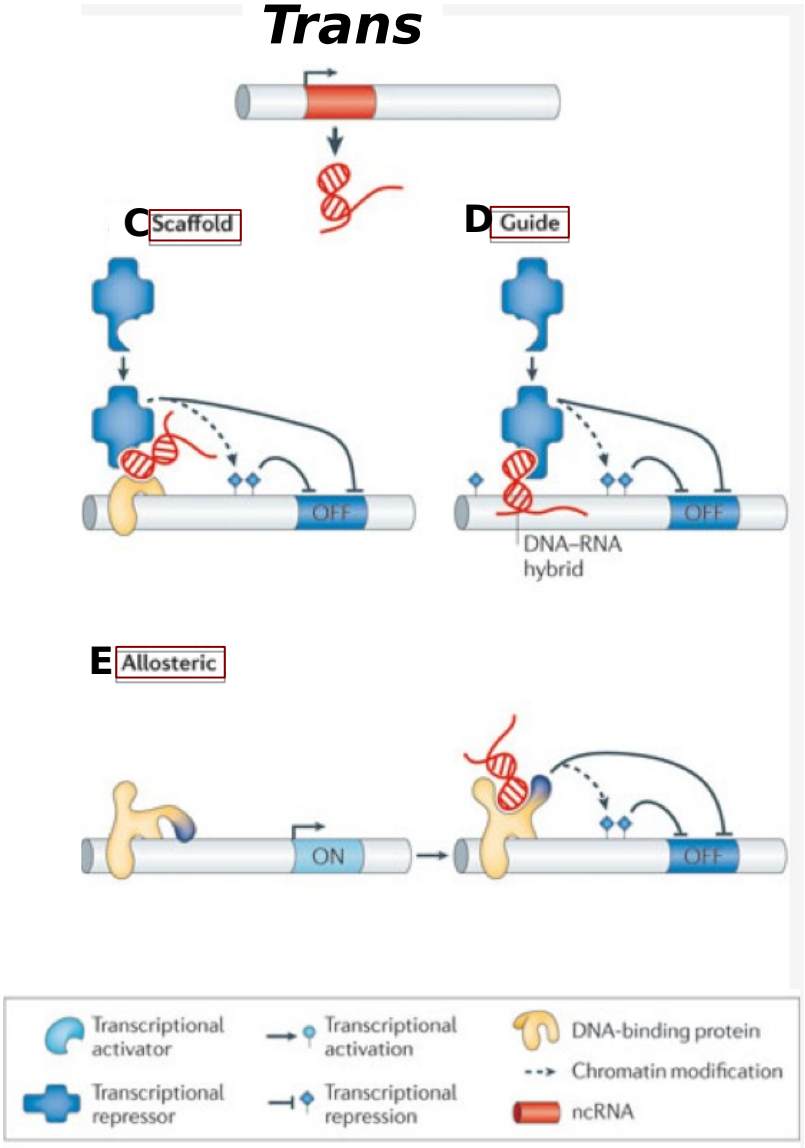
A. Transcription in *cis* displaces DNA-bound factors that inhibit (left) or activate (right) transcription of a neighbouring gene

B. Nascent ncRNA transcripts function as tethers for chromatin-modifying complexes and/or transcriptional regulators, which can have either activating (left) or repressive (right) activities



Potential molecular mechanisms of long noncoding RNAs

lncRNAs)

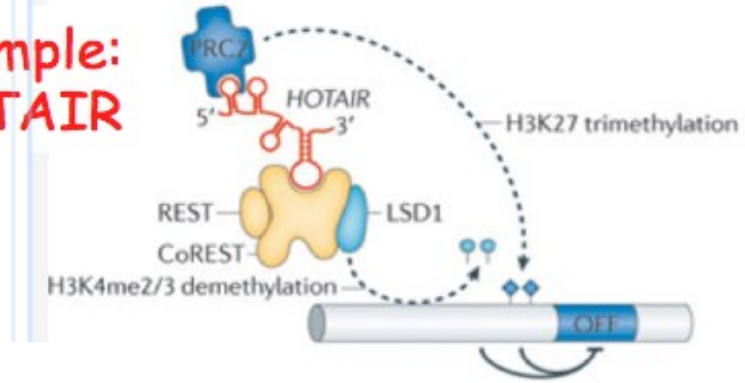


C. Trans-acting ncRNAs can serve as platforms for the assembly of protein complexes. Target sites are specified by DNA-binding proteins

D. *trans* ncRNAs specify target sites by forming
hybrids with complementary DNA sequences, and thus recruit chromatin modifiers and transcriptional regulator

E. lncRNAs also modulate the activity of protein
co
ch

Example: HOTAIR



Using CLIP to identify RNAs bound to proteins

UV x-link

↓

solate RNA/
rotein
complexes

↓

IP

↓

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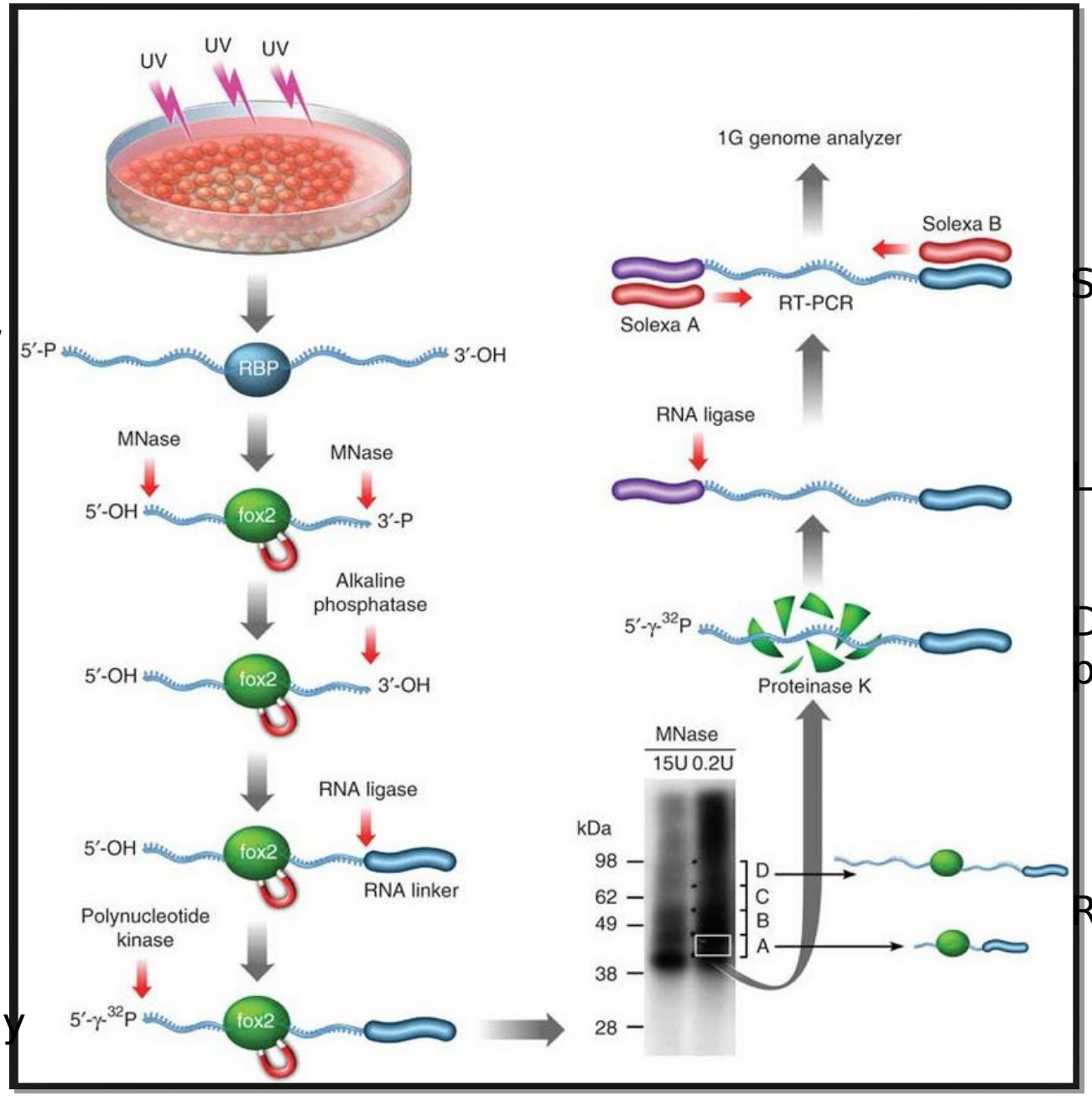
inkers

↓

adioactively
abel



Solexa sequenc

↑

igate 5’ adapte

↑

Digest bound
rotein

↑

Resolve on gel

Reading:

Regulation of chromatin by histone modifications
Bannister AJ and Kouzarides T
Cell Res., 2011, Vol 21: 381-395.

Chromatin modifications and their function.
Kouzarides T
Cell, 2007, Vol 128: 693-705.

Recommended:

Genome regulation by long noncoding RNAs.
Rinn JL and Chang HY
Annu. Rev. Biochem., 2012, Vol 81: 145-166